LAB FOUR PLANT PIGMENTS AND PHOTOSYNTHESIS

OVERVIEW

In this lab you will:

1. separate plant pigments using chromatography, and
2. measure the rate of photosynthesis in isolated chloroplasts using the dye DPIP.

The transfer of electrons during the light-dependent reactions of photosynthesis reduces DPIP, changing it from blue to colorless.

OBJECTIVES

Before doing this lab you should understand:

- how chromatography separates two or more compounds that are initially present in a mixture;
- the process of photosynthesis;
- the function of plant pigments;
- the relationship between light wavelength and photosynthetic rate; and
- the relationship between light intensity and photosynthetic rate.

After doing this lab you should be able to:

- separate pigments and calculate their R_f values;
- describe a technique to determine photosynthetic rates;
- compare photosynthetic rates at different light intensities or different wavelengths of light using controlled experiments; and
- explain why the rate of photosynthesis varies under different environmental conditions.

EXERCISE 4A: Plant Pigment Chromatography

Paper chromatography is a useful technique for separating and identifying pigments and other molecules from cell extracts that contain a complex mixture of molecules. The solvent moves up the paper by capillary action, which occurs as a result of the attraction of solvent molecules to the paper and the attraction of solvent molecules to one another. As the solvent moves up the paper, it carries along any substances dissolved in it. The pigments are carried along at different rates because they are not equally soluble in the solvent and because they are attracted, to different degrees, to the fibers in the paper through the formation of intermolecular bonds, such as hydrogen bonds.

Beta carotene, the most abundant carotene in plants, is carried along near the solvent front because it is very soluble in the solvent being used and because it forms no hydrogen bonds with cellulose. Another pigment, xanthophyll, differs from carotene in that it contains oxygen. Xanthophyll is found further from the solvent front because it is less soluble in the solvent and has been slowed down by hydrogen bonding to the cellulose. Chlorophylls contain oxygen and nitrogen and are bound more tightly to the paper than are the other pigments.

Chlorophyll *a* is the primary photosynthetic pigment in plants. A molecule of chlorophyll *a* is located at the reaction center of photosystems. Other chlorophyll *a* molecules, chlorophyll *b*, and the carotenoids (that is, carotenes and xanthophylls) capture light energy and transfer it to the chlorophyll *a* at the reaction center. Carotenoids also protect the photosynthetic system from the damaging effects of ultraviolet light.

Procedure

Your teacher will demonstrate the apparatus and techniques used in paper chromatography. Here is a suggested procedure, illustrated in Figure 4.1:

1. Obtain a 50-ml graduated cylinder that has 1 cm of solvent in the bottom. The cylinder is tightly stoppered because this solvent is volatile, and you should be careful to keep the stopper on as much as possible.
2. Cut a piece of filter paper that will be long enough to reach the solvent. Cut one end of this filter paper into a point. Draw a pencil line 1.5 cm above the point.
3. Use a coin to extract the pigments from spinach leaf cells. Place a small section of leaf on the top of the pencil line. Use the ribbed edge of the coin to crush the cells. Be sure that the pigment line is on top of the pencil line. You should repeat this procedure 8 to 10 times, being sure to use a new portion of the leaf each time.
4. Place the chromatography paper in the cylinder so that the pointed end is barely immersed in the solvent. *Do not allow the pigment to be in the solvent.*
5. Stopper the cylinder. When the solvent is about 1 cm from the top of the paper, remove the paper and *immediately* mark the location of the solvent front before it evaporates.
6. Mark the bottom of each pigment band. Measure the distance each pigment migrated from the bottom of the pigment origin to the bottom of the separated pigment band. In Table 4.1 record the distance that each front, including the solvent front, moved. Depending on the species of plant used, you may be able to observe 4 or 5 pigment bands.

Figure 4.1

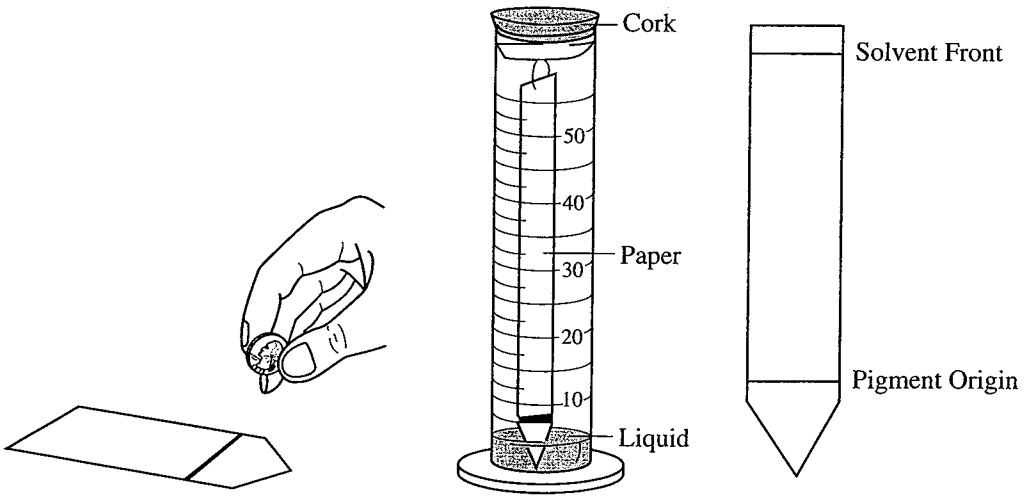


Table 4.1

Distance Moved by Pigment Band (millimeters)

Band Number	Distance (mm)	Band Color
1.		
2.		
3.		
4.		
5.		

Distance Solvent Front Moved _____ (mm)

Analysis of Results

The relationship of the distance moved by a pigment to the distance moved by the solvent is a constant called $\mathbf{R_f}$. It can be calculated for each of the four pigments using the following formula:

$$\mathbf{R_f} = \frac{\textbf{distance pigment migrated (mm)}}{\textbf{distance solvent front migrated (mm)}}$$

Record your $\mathbf{R_f}$ values in Table 4.2.

Table 4.2

_____ = R_f for Carotene (yellow to yellow orange)

_____ = R_f for Xanthophyll (yellow)

_____ = R_f for Chlorophyll *a* (bright green to blue green)

_____ = R_f for Chlorophyll *b* (yellow green to olive green)

Topics for Discussion

1. What factors are involved in the separation of the pigments?

2. Would you expect the R_f value of a pigment to be the same if a different solvent were used? Explain.

3. What type of chlorophyll does the reaction center contain? What are the roles of the other pigments?

EXERCISE 4B: Photosynthesis/The Light Reaction

Light is a part of a continuum of radiation, or energy waves. Shorter wavelengths of energy have greater amounts of energy. For example, high-energy ultraviolet rays can harm living tissues. Wavelengths of light within the visible part of the light spectrum power photosynthesis.

When light is absorbed by leaf pigments, electrons within each photosystem are boosted to a higher energy level, and this energy is used to produce ATP and to reduce NADP to NADPH. ATP and NADPH are then used to incorporate CO_2 into organic molecules, a process called **carbon fixation.**

Design of the Exercise

Photosynthesis may be studied in a number of ways. For this experiment a dye-reduction technique will be used. The dye-reduction experiment tests the hypothesis that light and chloroplasts are required for the light reactions to occur. In place of the electron acceptor, NADP, the compound DPIP (2,6-dichlorophenol-indophenol), will be substituted. When light strikes the chloroplasts, electrons boosted to high energy levels will reduce DPIP. It will change from blue to colorless.

In this experiment chloroplasts are extracted from spinach leaves and incubated with DPIP in the presence of light. As the DPIP is reduced and becomes colorless, the resultant increase in light transmittance is measured over a period of time using a spectrophotometer. The experimental design matrix is presented in Table 4.3.

Lab 3: Photosynthesis

INTRODUCTION:

Photosynthesis is the process by which plants take carbon dioxide from the atmosphere, add water, and use the energy of sunlight to produce sugar. The overall equation is:

$$6CO_2 + 6H_2O \text{ --> } C_6H_{12}O_6 + 6O_2$$

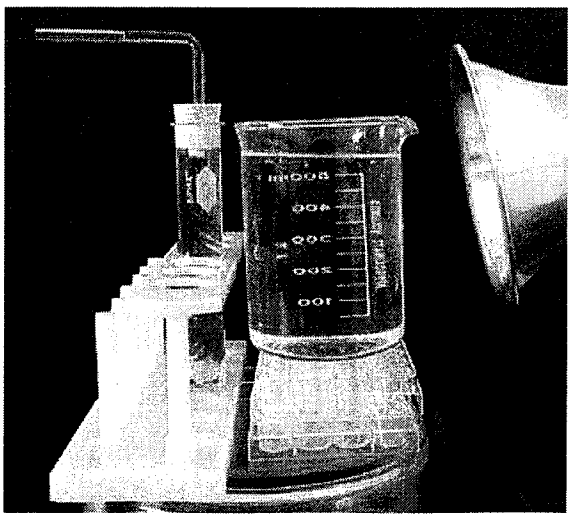
Part 1: Measuring the Rate of Photosynthesis

Materials:

- test tube
- stopper fitted with glass tubing
- Aquarium plant cuttings
- 3% sodium bicarbonate solution
- beaker with water
- lamp

Procedures:

1. Place a generous quantity of aquarium plants with the cut side up in a test tube with a rubber stopper fitted with a piece of glass tubing.
2. Add 3% sodium bicarbonate solution, enough so that when the stopper is fitted onto the tube the solution fills the tubing to about 1/4 of the length of the horizontal portion. Note the initial value on the pipette.
3. Place a beaker of plain water next to the plant tube to serve as a heat absorber. Place a lamp next to the beaker. The tube, beaker, and lamp should be as close to one another as possible. See picture below.



4. Turn on the lamp. As soon as the edge of the solution in the tubing begins to move, time the reaction every 3 minutes for 18 minutes. Be careful not to bump the tubing or to readjust the stopper, or your readings will be altered.

	Reading
Time	
0	
3	
6	
9	
12	
15	
18	

5. Calculate the net photosynthesis in mm/min. (Divide the mm of movement by 10 minutes.)

You may want to calculate an AVERAGE rate as well as INTERVAL rates

Part 2: Creating your own photosynthesis lab

1. With the members of your lab group brainstorm variables which may affect the rate of photosynthesis.
2. Form hypotheses about your variables.
3. Design an experiment to test your hypotheses. Discuss materials you may need and ask me if it is reasonable to try for your experimental lab.
4. Groups must devise a protocol by the end of today, less a 50% deduction on all members' lab reports.